UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: May 8, 2012

SUBJECT: Tefluthrin, Immunotoxicity study in mice

PC Code: 128912
Decision No.: 462654
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0056299
MRID No.: 48756301

DP Barcode: 400351
Registration No.: N/A
Regulatory Action: N/A
Submission No.: N/A
CAS No.: 79538-32-2
40 CFR: N/A

FROM: **Yung G. Yang, Ph.D.**
Risk Assessment Branch VI
Health Effects Division (7509 P)

THROUGH: **Felecia Fort, Chief**
Risk Assessment Branch VI
Health Effects Division (7509 P)

TO: **Bewanda Alexander**
Insecticide Branch
Registration Division (7505P)

I. CONCLUSIONS

The immunotoxicity study in mice for Tefluthrin (MRID 48756301) has been reviewed. It is classified as acceptable/guideline and satisfies guideline requirements for an immunotoxicity study (OPPTS 870.7800).

1 of 11

II. BACKGROUND and ACTION REQUESTED

An immunotoxicity study on Tefluthrin (MRID 48756301) has been submitted. RAB VI was asked to review and prepare a DER for this study.

III. RESULTS AND DISCUSSION

The immunotoxicity study in mice for Tefluthrin (MRID 48756301) has been reviewed. The DER is attached and an executive summary is as follows:

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 48756301), tefluthrin (96.3%, a.i.) was administered to female CD-1mice (10/group) in diets containing 0, 100, 200 or 400 ppm (equivalent to 0, 16, 31, 62 mg/kg/day, respectively) for 28 consecutive days. The positive control group consisted of 10 females received 10 mg/kg/day of cyclophosphamide (CPH) by oral gavage for 28 consecutive days. On Day 25, all animals in all groups received a single intravenous injection of the antigen, sheep red blood cells (SRBC, 0.25 mL/$2x10^8$ cells/animal). The animals were monitored for mortality and for treatment related symptoms daily. Body weights were recorded twice weekly from Day (-7) until necropsy, and food consumption were measured and recorded daily. On Day 29, blood samples were collected from the retro orbital sinus under isoflurane anesthesia for evaluation of T-cell dependent antibody response (TDAR). After collecting blood samples, all animals were killed by carbon dioxide inhalation and severance of major blood vessels to exsanguinate. All animals were subjected to a necropsy. Liver (with gall bladder), spleen and thymus were weighed from all animals.

There were no deaths during the study period. There were no treatment related clinical signs and symptoms in 100 and 200 ppm groups. In the 400 ppm group, one animal had a hunched appearance from Day 4 to the end of the treatment period; piloerection was also observed in this animal on Day 4 and 5. Animals in the 400 ppm group had reduced mean body weights and body weight gains. On Day 28, the overall mean body weight gain in this group was 82% lower than the controls. There were no differences in food consumption and organ weights among treated and the control groups. The positive control group that received (CPH) had no difference in body weights, food consumption and organ weights when compared with control group.

The systemic toxicity NOAEL is 200 ppm (equivalent to 31 mg/kg/day); the systemic LOAEL was 400 ppm (equivalent to 62 mg/kg/day) based on lower body weights and body weight gains.

There were no differences in anti-SRBC IgM antibody response in treated and the control groups by ELISA test. There was a high inter-individual variability noted in all the treatment groups as well as in the control group. Evaluation of individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC IgM response. Animals in the positive control group showed statistically significant ($p<0.01$) decreases in anti-SRBC IgM response to a challenge with SRBCs compared to the vehicle control group. This confirmed the ability of the test

system to detect immunosuppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for tefluthrin does not reveal any evidence of treatment-related effects on the immune system. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under HED guidance, a NK cells activity assay is not required at this time.

Under conditions of this study, the immunotoxicity NOAEL is 400 ppm (equivalent to 62 mg/kg/day). The LOAEL was not established (>400 ppm).

This immunotoxicity study is classified **acceptable/guideline** and satisfies guideline requirements for an immunotoxicity study (OPPTS 870.7800) in mice.

EPA Reviewer: Khin Swe Oo, M.D., DABT **Signature:**
Toxicology and Epidemiology Branch, Health Effects Division (7509P) Date: 5/3/2012
EPA Secondary Reviewer: Yung G. Yang, Ph.D. **Signature:**
Risk Assessment Branch VI, Health Effects Division (7509P) Date: 5/3/2012

TXR #: 0056299

DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity – 28 Day Oral (Dietary) Study; OPPTS 870.7800

PC CODE: 128912 **DP BARCODE:** D400351

TEST MATERIAL (PURITY): Tefluthrin (96.3%)

SYNONYMS: Cyclopropanecarboxylic acid, 3-[(1Z)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethyl-, (2,3,5,6-tetrafluoro-4-methylphenyl)methyl ester.

CITATION: Donald L., Marr C., (2011).Tefluthrin – A 28 Day Immunotoxicity Study by Oral (Dietary) Administration in Mice using Sheep Red Blood Cells as the Antigen. Charles River Laboratories, Tranent, Edinburgh EH 33 2NE, UK. Study number 520094, November 24, 2011. MRID 48756301. Unpublished.

SPONSOR: Syngenta Crop Protection, LLC; 410 Swing Road, PO Box 18300, Greensboro, NC 27419-8300, USA.

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The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for tefluthrin does not reveal any evidence of treatment-related effects on the immune system. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under HED guidance, a NK cells activity assay is not required at this time.

Under conditions of this study, the immunotoxicity NOAEL is 400 ppm (equivalent to 62 mg/kg/day). The LOAEL was not established (>400 ppm).

This immunotoxicity study is classified **acceptable/guideline** and satisfies guideline requirements for an immunotoxicity study (OPPTS 870.7800) in mice.

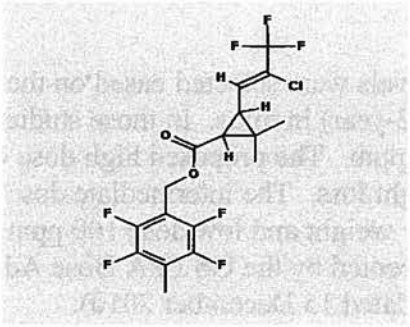
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Tefluthrin

Description:	Off-white crystalline solid
Lot/Batch #:	1148
Purity:	96.3 %
Compound Stability:	Stable for 8 days at -20°C.
CAS # of TGAI:	79538-32-2



2. Vehicle and/or positive control: Rat and mouse (modified) No. 1 Diet SQC Expanded (Ground) for vehicle control group. Positive control group received cyclophosphamide monohydrate (10mg/kg/day) 5 ml/kg/day, Lot/Batch no. 079K1569, purity 100.5% for 28 consecutive days.

3 Test animals:

Species:	Mouse, female	
Strain:	Cr:l:CD-1(ICR)	
Age/weight at study initiation:	Approx. 8 weeks, 22-23 grams at initiation of treatment	
Source:	Charles River UK Limited, Margate, Kent	
Housing:	Housed 2 or 3 per cage in suspended polypropylene cages.	
Diet:	Rat and Mouse No. 1 Expanded (Ground) diet, *ad libitum*	
Water:	Public water supply (Scottish water, Edinburgh, UK), *ad libitum*	
Environmental conditions:	**Temperature:**	18-23°C
	Humidity:	30-84%
	Air changes:	15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	23 days	

B. STUDY DESIGN:

1. In life dates – Experiment Start: May 27, 2011 End: July 1, 2011

2. Animal assignment: Animals were removed from transport boxes in a random order and allocated to cages on racks. Vehicle control and positive control groups were housed on separate racks.

Table 1. Study Design[a]				
Group No.	Dietary Treatment	Dietary Dose (ppm)	Achieved dosage (mg/kg/day)	No. of animals
1	Control	0	0	10
2	Positive control	0	10[b]	10
3	Tefluthrin	100	16	10
4	Tefluthrin	200	31	10
5	Tefluthrin	400	62	10

- [a] Data obtained from pages 14 and 31 of the study report.
- [b] Cyclophosphamide (CPH) was given to positive control group by oral gavage 10mg/kg/day (5ml/kg) for 28 days.

3. **Dose selection:** The dose levels were selected based on the results from previous dietary toxicity studies (4-week and 2-year) in mice. In those studies, slight decreases in body weight were observed at 400 ppm. The proposed high dose 400 ppm was anticipated to produce a minimal body weight loss. The intermediate dose 200 ppm was anticipated to have minimal effects on body weight and low dose 100 ppm was expected to have no effects. The dosages were accepted by the US EPA Dose Adequacy and Review Team (DART, TXR No. 0055384, dated 15 December 2010).

4. **Diet preparation and analysis:** Diet was prepared weekly by preparing the premix and mixing it with an appropriate amount of Rat and Mouse No. 1 Diet to achieve the final concentration. Prepared diet was stored at ambient temperature. Homogeneity and stability were tested from top, middle and bottom samples of each group. During the study, samples of treated food were analyzed for stability and concentration.

Results of Diet Analysis

Homogeneity analysis: C.V. (the difference between the 3 samples taken from each diet at each time point) was less than 10%.

Stability analysis: Stable for 8 days at -20°C.

Concentration analysis: Concentration analysis was done two times. For 100, 200 and 400 ppm groups, percentage difference from theoretical concentration were (-9.7, -11.3) %, (-6, -8) %, (-5.8, -10.3) % respectively.

5. **Statistics:** A parametric ANOVA test was used to analyze body weights, cumulative body weight gain, food consumption and antibody data. Dunnett's t-test was used for pairwise comparisons between control group and treated groups. Student's t-test was used to compare the vehicle and positive control groups. Organ weights were analyzed with ANCOVA using terminal body weight as covariate. All statistical tests were two-sided and performed at the 5% and 1% significance level. The statistical tests applied were considered appropriate.

C. METHODS:

1. **Observations:**
The animals were monitored for morbidity and for treatment related symptoms daily. Positive control group animals were examined at least 3 times daily.

2. **Body weight:**
Body weights were recorded twice weekly from Day -7 until necropsy

3. **Food/water consumption and compound intake:**
Food consumption were measured and recorded daily. Water consumption was monitored weekly.

4. **Sacrifice and pathology**
On Day 29, all animals were sacrificed by carbon dioxide inhalation and severance of major blood vessels to exsanguinate.

   a. **Gross necropsy:** all animals were subjected to a necropsy which includes complete external and internal examinations. Thymus, spleen, and liver with gall bladder were weighed.

   b. **Tissue preparation/histopathology:** Tissues have been saved for possible evaluation.

5. **Immunotoxicity:**

a. Anti-SRBC IgM Enzyme-Linked Immunosorbent Assay (ELISA): Animals were exposed to the test substance for 28 days except in the treatment groups. On Day 25 all animals were injected intravenously with sheep red blood cell (SRBC, $2x10^8$ /animal; 0.25mL/animal). On day 29 (peak day of IgM response), 0.3 ml of whole blood was collected from the orbital sinus under isoflurane anesthesia. The primary IgM response to sheep erythrocytes was measured using a mouse anti-SRBC IgM ELISA kit (life Diagnostics, Inc. Catalogue no. 4200-1).

b. Natural Killer (NK) Cells Activity Assay: NK cells assay was not performed. Data provided to EPA DART team, from the subchronic, chronic and reproductive studies of tefluthrin in the rat, mouse and dog did not provide any evidence of immunotoxic effect.

II. RESULTS:

A. OBSERVATIONs:

1. **Clinical signs of toxicity:** There were no treatment related signs and symptoms in 100 and 200 ppm groups. In 400 ppm group, one animal had a hunched appearance from Day 4 to the end of the treatment period. Piloerection was also observed in this animal on Day 4 and 5.

2. **Mortality**: There were no deaths during the study period.

8

B. Body weight and weight gain: Animals in the 400 ppm group had reduced mean body weights and body weight gains. On Day 28, the overall mean body weight gains in this group were 82% lower than the controls (Table 2).

Table 2. Mean body weights and body weight gains during 28 days of treatment

Dose Group (ppm)	Body weights (g ± SD)				Total weight gain (Days 0-28)	
	Day 0	Day14	Day 21	Day 28	g ± SD	% of control
0	29.1±2.1	29.6±2.3	28.6±2.0	30.2±1.7	1.1±1.3	--
100	27.2±1.7	26.9±2.1	27.0±1.4	28.6±1.9	1.5±1.3	136%
200	28.0±2.9	28.3±3.1	27.9±3.1	29.5±2.2	1.6±2.4	145%
400	27.0±2.7	**25.2[b]±2.3**	26.4±2.3	**27.2[a] ±2.7**	**0.2±1.3**	**18%**
Positive control	**26.8[a]±2.6**	**27.2[a]±2.5**	27.5±3.1	28.0±3.0	1.2±1.6	109%

Data obtained from pages (36) in the study report
[a] Statistically different (p <0.05) from the control.
[b] Statistically different (p <0.01) from the control.
10 animals in each group.

C. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** No differences in food consumption in all groups.

2. **Water consumption:** No differences between groups.

3. **Compound consumption:** included in Table 1.

4. **Food efficiency:** Did not report.

D. GROSS NECROPSY: No abnormal findings noted at necropsy.

1. **Organ weight:** There were no differences in organ weight of treated groups compared with the control group.

Table 3: Mean terminal body weights and Organ weights, Mean±SD

Dose Group (ppm)	Body weight (g)	Liver (g)	Spleen (g)	Thymus (g)
Vehicle Control	29±2	1.80±0.35	0.159±0.044	0.051±0.008
100	28±2	1.59±0.18	0.147±0.032	0.048±0.007
200	28±2	1.66±0.21	0.154±0.028	0.046±0.012
400	**26** ** **±3**	1.54±0.25	0.154±0.034	0.045±0.015
Positive Control	27.0±2.9	1.64±0.30	0.132±0.034	0.049±0.011

n = 10 animals per dose group
Information was obtained from pages 49 and 50 of the study report
** p<0.01

2. **Histology**: Not reported.

9

E. IMMUNOTOXICITY TESTS:

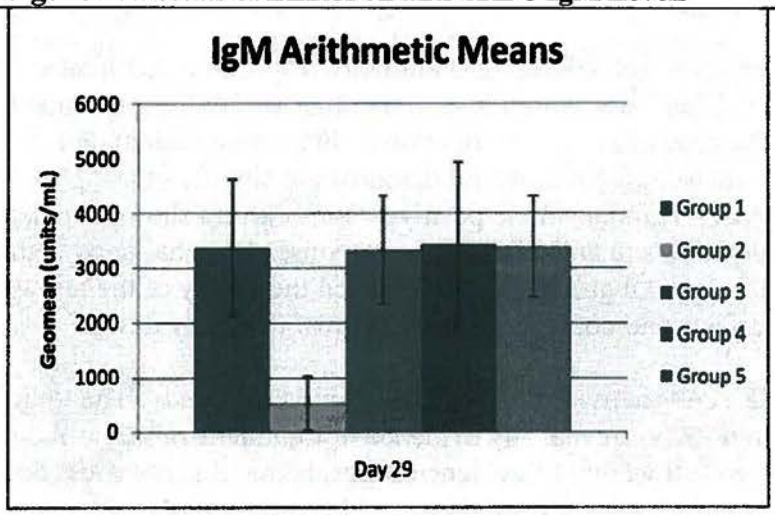
1. **Enzyme-linked immunosorbent assay (ELISA):** There were no differences in anti-SRBC IgM levels among treated groups and the vehicle control group (Table 4 and Figure 1). There was a high inter-individual variability noted in all the treatment groups as well as in the control group. Evaluation of individual animal data of this study did not show any trend or distribution that would demonstrate a significant suppression of serum anti-SRBC IgM antibody response. Animals in the positive control group showed a statistically significant ($p<0.01$) decrease of anti-SRBC IgM response to a challenge with SRBCs compared to the vehicle control group. This confirmed the ability of the test system to detect immunosuppressive effects and confirmed the validity of the study design.

Table 4: Results of ELISA on anti-SRBC IgM Levels

Group (ppm)	IgM units / mL (mean ± SD)
1. Vehicle control (0)	3364 ± 1270
2. Positive control (Cyclophosphamide 10mg/kg/day)	**528 ** ± 492**
3. 100	3330 ± 988
4. 200	3421 ± 1519
5. 400	3394 ± 928

Data extracted from page 93 of study report.
** $p<0.01$.

Figure 1: Results of ELISA on anti-SRBC IgM Levels



Data extracted from page 92 of study report.

2. **NK cell activity assay:** Did not perform NK cell assay.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The report concluded that animals received 100, 200 and 400 ppm of tefluthrin had similar anti-SRBC IgM levels with the control group. Under the conditions of the study, after 28 consecutive days of dietary treatment to female

10

CD-1 mice with up to 400 ppm tefluthrin, there was no evidence of an immunosuppressive effect on the humoral immune system.

B. REVIEWER COMMENTS: In an immunotoxicity study (MRID 48756301), tefluthrin (96.3%) was administered to female CD-1mice (10/group) in diets containing 0, 100, 200 or 400 ppm for 28 consecutive days. The positive control group consisted of 10 females received 10 mg/kg/day of cyclophosphamide (CPH) by oral gavage for 28 consecutive days. On Day 25, all animals in all groups received a single intravenous injection of the antigen, sheep red blood cells (SRBC, 0.25 mL/2x10^8 cells/animal). On Day 29, blood samples were collected from the retro orbital sinus under isoflurane anesthesia for anti-SRBC antibody analysis.

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C. STUDY DEFICIENCIES: No major deficiencies were found.

11